REMARKS

Status of the claims:

With the above amendments, claims 1-24 and 27-41 have been canceled and claims 42-65 have been added. Thus, claims 25, 26, and 42-65 are pending and ready for further action on the merits. No new matter has been added by way of the above amendments. Support for new claims 42-65 can be found at page 19, lines 18-19, page 6, line 25 to page 7, line 4, page 16, line 16, page 19, lines 8-10, page 3, lines 15-19, page 11, lines 1-7, page 37, line 28, page 17, lines 1-2, page 5, line 4, and page 13, lines 1-3, page 2, lines 28-29, the bottom of page 1 and page 3, line 11 *et seq*. Reconsideration is respectfully requested in light of the following remarks.

Declaration

The Examiner asserts that the 37 CFR §1.132 declaration filed with Applicants' previous response attesting to the public availability of the genes in the specification was not attached to the reply. Applicants again submit herewith the 37 CFR §1.132 declaration that was filed on January 22, 2003.

[redacted]

Specification Objections

The Examiner objects to the presence of web sites in the specification on page 47, line 7 and page 47, line 30. Applicants have amended the written description to delete these web links and insert the literature references to which these web links refer. Withdrawal of the objections is warranted and respectfully requested.

Claim Objections

Claim 21 has been objected to for its dependency. Claim 21 has been deleted. Thus, the objection is moot. Withdrawal of the objection is warranted and respectfully requested.

Rejections under 35 USC §112, second paragraph

Claims 1-10, 17-22, 28-30, 33, and 38-41 are rejected under 35 USC §112, second paragraph as being indefinite.

Claims 1, 2 and 40 have been rejected for reciting the phrase: "functional coupling of the oxidation and reduction of substrates". This phrase no longer appears in the claims and thus the rejection is moot. Withdrawal of the rejection is warranted and respectfully requested.

However, for the Examiner's benefit "functional coupling" refers to a coupling between the oxidation and reduction

reactions of substrates. The oxidation and reduction reactions are mediated by NAD/NADH-linked dehydrogenases and NADP/NADPH-linked dehydrogenases. Applicants direct the Examiner's attention to page 6, lines 27-33 wherein it is described that pyridine nucleotide-linked dehydrogenases are one class of enzymes that functionally couple oxidation and reduction of substrates.

The Examiner has rejected claims 1 and 2 for reciting the phrase "more efficiently". This phrase no longer appears in the claims and thus the rejection is moot. Withdrawal of the rejection is warranted and respectfully requested.

The Examiner has rejected claim 2 for the recitation of the phrase "under different physiological conditions than said natural promoter". This phrase no longer appears in the claims and thus the rejection is moot. Withdrawal of the rejection is warranted and respectfully requested.

Claims 1 and 2 have been rejected for the phrases "cheaper process", "a higher specific rate", "a higher volumetric rate", "higher specific rate", "higher yield of product from carbohydrate", "smaller amounts of unwanted side products", and "a smaller oxygen requirement". These phrases no longer appear

in the claims and thus the rejection is moot. Withdrawal of the rejection is warranted and respectfully requested.

The Examiner has rejected "one or more products" in claim 40. Claim 40 has been canceled so the rejection is moot. Withdrawal of the rejection is warranted and respectfully requested.

Rejections under 35 USC §112, first paragraph

Claims 1-10, 17-22, 28-30, 33, and 38-41 are rejected under 35 USC §112, first paragraph as allegedly lacking description.

Rejections under 35 USC §112, first paragraph

Claims 1-10, 17-22, 28-30, 33, and 38-39 are rejected under 35 USC §112, first paragraph as allegedly lacking description and lacking full enablement.

Claims 1-10, 17-22, 28-30, 33, and 38-41 have been canceled so the rejections are moot with respect to those claims.

Applicants have presented a series of new claims (i.e., claims 42-65) in the instant response. Applicants submit the following comments regarding these new claims.

In a telephonic Interview that was held with the Examiner and her supervisor on July 17, 2003, the Examiner indicated that at a minimum for Applicants to procure a generic product claim,

it would have to be shown that many of the genes that encode the enzymes listed in, e.g. claim 43, were known at the time of filing the Application. The Examiner also indicated that "method" claims might better define Applicants' invention. With these points in mind, Applicants have amended the claims to present "method" claims.

Moreover, Applicants assert that many of the genes that encode the proteins recited in the claims (e.g., claim 43) were known at the time of filing the instant invention. Accordingly, Applicants provide Exhibit 1 providing examples of cloned genes that were known (and are claimed in e.g. claim 43) at the time of filing the instant application. The following list summarizes the enzymes listed in Exhibit 1 for which cloned genes were known as of the filing date of the instant invention.

1) glutamate dehydrogenase,

2) malic enzyme,

3) aldehyde dehydrogenase,

4) alcohol dehydrogenase,

5) malate dehydrogenase,

6) glycerol-3-phosphate dehydrogenase,

7) glyceraldehyde-3-phosphate dehydrogenase, and

8) ferredoxin reductase

Please also note that the dates that these genes were known was prior to the filing date of the instant invention.

Furthermore, to the degree that the Examiner might suggest that a large number of species of genes must be described, Applicants submit that the disclosure at pages 24 and 25 of the specification describes how to obtain cloned genes starting from either a first cloned gene or a purified protein. Exhibit 2 shows examples of purified proteins including xylose-1-dehydrogenase and orotate reductase (see e.g. claim 43) to which the methods described at pages 24 and 25 of the specification might be applied.

Applicants submit that with what was known in the prior art and with the instant written description, Applicants of the instant invention did have possession of the full scope of the claimed invention at its time of filing. Moreover, Applicants submit that the full scope of the invention can be practiced without undue experimentation. Thus, any written description rejection or enablement rejection over the instant claims is inapposite.

Accordingly, with the above remarks and amendments, it is believed that the claims, as they now stand, define patentable subject matter such that passage of the instant invention to

allowance is warranted. A Notice to that effect is earnestly solicited.

If any questions remain regarding the above matters, please contact Applicant's representative, T. Benjamin Schroeder (Reg. No. 50,990), in the Washington metropolitan area at the phone number listed below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Mark J. Nuell #36,623

GMM/DRN/TBS/jmb/mua

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

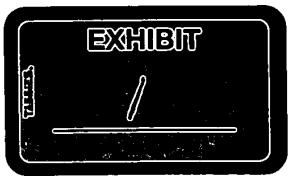
Attachment: Copy of Declaration under 37 C.F.R. § 1.132 filed January 22, 2003.

Exhibit 1: List of Genes available at time of filing the application

Exhibit 2: Examples of Purified Proteins

[redacted]

Examples of pairs of genes encoding pairs of dehydrogenases with different specificities for NAD and NADP



1. Glutamate dehydrogenase.

☐ L4 ANSWER 1 OF 5 MEDLINE on STN

97113544 MEDLINE Full Text

The NAD(P)H-utilizing glutamate dehydrogenase of Bacteroides thetaiotaomicron belongs to enzyme family I, and its activity is affected by trans-acting gene(s) positioned downstream of gdhA.

Author

Baggio L; Morrison M

Corporate Source

Department of Animal Sciences, University of Nebraska, Lincoln 68583, USA.

Source

JOURNAL OF BACTERIOLOGY, (1996 Dec) 178 (24) 7212-20. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

Previous studies have suggested that regulation of the enzymes of ammonia assimilation in human colonic Bacteroides species is coordinated differently than in other eubacteria. The **gene encoding** an **NAD** (P)H-dependent **glutamate dehydrogenase** (gdhA) in Bacteroides thetaiotaomicron was cloned and expressed in Escherichia coli by mutant complementation from the recombinant plasmid pANS100. Examination of the predicted GdhA amino acid sequence revealed that this enzyme possesses motifs typical of the family I-type hexameric GDH proteins. Northern blot analysis with a gdhA-specific probe indicated that a single transcript with an electrophoretic mobility of approximately 1.6 kb was produced in both B. thetaiotaomicron and E. coli gdhA+ transformants. Although gdhA transcription was unaffected, no GdhA enzyme activity could be detected in E. coli transformants when smaller DNA fragments from pANS100, which contained the entire gdhA gene, were analyzed. Enzyme activity was restored if these E. coli strains were cotransformed with a second plasmid, which contained a 3-kb segment of DNA located downstream of the gdhA coding region. Frameshift mutagenesis within the DNA downstream of gdhA in pANS100 also resulted in the loss of GdhA enzyme activity. Collectively, these results are interpreted as evidence for the role of an additional gene product(s) in modulating the activity of GDH enzyme activity. Insertional mutagenesis experiments which led to disruption of the gdhA gene on the B. thetaiotaomicron chromosome indicated that gdhA mutants were not glutamate auxotrophs, but attempts to isolate similar mutants with insertion mutations in the region downstream of the gdhA gene were unsuccessful.

☐ L6 ANSWER 1 OF 7 MEDLINE on STN

96180651 MEDLINE Full Text

Nucleotide sequence and expression of the gene encoding NADP +- dependent

[redacted]

glutamate dehydrogenase (gdhA) from Agaricus bisporus.

Author

Schaap P J; Muller Y; Baars J J; Op den Camp H J; Sonnenberg A S; van Griensven L J; Visser J

Corporate Source

Section Molecular Genetics of Industrial Microorganisms, Wageningen Agricultural University, The Netherlands.

Source

MOLECULAR AND GENERAL GENETICS, (1996 Feb 25) 250 (3) 339-47. Journal code: 0125036. ISSN: 0026-8925.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

The **gene encoding NADP** +-dependent ***glutamate*** **dehydrogenase** (gdhA) was isolated from an Agaricus bisporus recombinant phage lambda library. The deduced amino acid sequence would specify a 457-amino acid protein that is highly homologous in sequence to those derived from previously isolated and characterized genes coding for microbial NADP+-GDH. The open reading frame is interrupted by six introns. None of the introns is located at either one of the positions of the two introns conserved in the corresponding open reading frames of the ascomycete fungi Aspergillus nidulans and Neurospora crassa. Northern analysis suggests that the A. bisporus gdhA gene is transcriptionally regulated and that, unlike the case in ascomycetes, transcription of this gene is repressed upon the addition of ammonium to the cu

Aldehyde dehdrogenase

☐ L12 ANSWER 1 OF 6 MEDLINE on STN

96134984 MEDLINE Full Text

Cloning and characterization of a gene (msdA) encoding methylmalonic acid semialdehyde dehydrogenase from Streptomyces coelicolor.

Author

Zhang Y X; Tang L; Hutchinson C R

Corporate Source

School of Pharmacy, University of Wisconsin, Madison 53706, USA. CONTRACT NUMBER: GM31925 (NIGMS)

Source

JOURNAL OF BACTERIOLOGY, (1996 Jan) 178 (2) 490-5. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

A homolog of the mmsA **gene** of Pseudomonas aeruginosa, which ***encodes*** methylmalonic acid semialdehyde dehydrogenase (MSDH) and is involved in valine catabolism in pseudomonads and mammals, was cloned and sequenced from Streptomyces coelicolor. Of the two open reading frames (ORFs) found, which are convergently transcribed and separated by a 62-nucleotide noncoding region, the deduced amino acid

[redacted]

sequence of the msdA ORF (homologous to mmsA) is similar to a variety of prokaryotic and eukaryotic **aldehyde** ***dehydrogenases*** that utilize **NAD** +, particularly to the MmsA protein from P. aeruginosa. No significant similarity was found between the deduced product of ORF1 and known proteins in the databases. An S. coelicolor msdA mutant, constructed by insertion of a hygromycin resistance **gene** (hyg) into the msdA **coding** region, lost the MSDH activity and the ability to grow in a minimal medium with valine or isobutyrate as the sole carbon source but grew on propionate. The msdA::hyg mutation was complemented by introduction of the msdA gene on a plasmid. When the S. coelicolor msdA gene was overexpressed in Escherichia coli under the control of the T7 promoter, a protein of 51-kDa, corresponding to the approximate mass of the predicted S. coelicolor msdA product (52.6 kDa), and specific MSDH activity were detected. These results strongly suggest that msdA indeed encodes the MSDH that is involved in valine catabolism in S. coelicolor.

Primary structures of alcohol and aldehyde dehydrogenase genes of Entamoeba histolytica.

Author

Samuelson J; Zhang W W; Kumar A; Descoteaux S; Shen P S; Bailey G

Corporate Source

Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.

Source

ARCHIVES OF MEDICAL RESEARCH, (1992) 23 (2) 31-3. Journal code: 9312706. ISSN: 0188-4409.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

Ethanol is the major metabolic product of glucose fermentation by the protozoan parasite E. histolytica under the anaerobic conditions found in the lumen of the colon. With the goal of finding new targets for anti-amebic drugs, the E. histolytica **NADP** (+)-dependent alcohol dehydrogenase gene (EhADH1; EC 1.1.1.2) and an **aldehyde dehydrogenase** gene (EhALDH1; EC 1.3.2) were cloned. The EhADH1 alcohol dehydrogenase **gene** ***encoded*** -39 kDa protein with 62 and 60% amino acid identities, respectively, with NADP(+)-dependent alcohol dehydrogenases of anaerobic bacteria Thermoanaerobium brockii and Clostridia beijerinckii. In contrast, EhADH1 showed a 15% amino acid identity with the closest human alcohol dehydrogenase. An EhADH1-glutathione-S-transferase fusion protein showed the expected NADP(+)-dependent alcohol dehydrogenase and NADPH-dependent acetaldehyde reductase activities. The enzymatic activities of the EhADH1 fusion protein were inhibited by pyrazole and 4-methyl pyrazole. The E. histolytica aldehyde dehydrogenase EhALDH1 **gene encoded** a 60 kDa protein, which showed a 36% amino acid identity over a 451 amino acid overlap with the human stomach aldehyde dehydrogenase (ALDH3).

Alcohol dehydrogenase

☐ L55 ANSWER 2 OF 17 MEDLINE on STN

96125263 MEDLINE Full Text

Cloning and overexpression in Escherichia coli of the genes encoding NAD - dependent alcohol dehydrogenase from two Sulfolobus species.

Author

Cannio R; Fiorentino G; Carpinelli P; Rossi M; Bartolucci S

Corporate Source

Dipartimento di Chimica Organica e Biologica, Universita degli Studi di Napoli Federico II, Italy.

Source

JOURNAL OF BACTERIOLOGY, (1996 Jan) 178 (1) 301-5. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

The **gene** adh **encoding** a **NAD** -dependent ***alcohol*** **dehydrogenase** from the novel strain RC3 of Sulfolobus sp. was cloned and sequenced. Both the adh gene from Sulfolobus sp. strain RC3 and the alcohol dehydrogenase gene from Sulfolobus solfataricus (DSM 1617) were expressed at a high level in Escherichia coli, and the recombinant enzymes were purified, characterized, and compared. Only a few amino acid replacements were responsible for the different kinetic and physicochemical features investigated.

☐ L14 ANSWER 1 OF 5 MEDLINE on STN

97352709 MEDLINE Full Text

Purification and sequence analysis of a novel NADP (H)-dependent type III alcohol dehydrogenase from Thermococcus strain AN1.

Author

Li D; Stevenson K J

Corporate Source

Department of Biological Sciences, The University of Calgary, Alberta, Canada.

Source

JOURNAL OF BACTERIOLOGY, (1997 Jul) 179 (13) 4433-7. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

An **NADP** (H)-dependent **alcohol dehydrogenase** was isolated from the hyperthermophilic archaeon Thermococcus strain AN1. This enzyme is a homotetramer with a subunit molecular weight of 46,700. The enzyme oxidizes a series of primary linear alcohols but not methanol. The pH and temperature optima with ethanol as the substrate are 6.8 to 7.0 and 85 degrees C, respectively. The enzyme readily reduced acetaldehyde with NADPH as the cofactor. The **gene encoding** this enzyme has been cloned and sequenced. An open reading frame of 1,218 bp, starting with ATG and ending with TGA, was identified and corresponded to 406 amino acids. Sequence comparisons show that this Thermococcus strain AN1 enzyme has significant homologies with enzymes from the newly defined type

III alcohol dehydrogenase family. Thermococcus strain AN1 alcohol dehydrogenase is the first archaeal enzyme belonging to this family.

Malate dehydrogenase

☐ L16 ANSWER 4 OF 7 MEDLINE on STN

95161707 MEDLINE Full Text

Expression of a single gene encoding microbody NAD -malate dehydrogenase during glyoxysome and peroxisome development in cucumber.

Author

Kim D J; Smith S M

Corporate Source

Institute of Cell and Molecular Biology, University of Edinburgh, UK.

Source

PLANT MOLECULAR BIOLOGY, (1994 Dec) 26 (6) 1833-41. Journal code: 9106343. ISSN: 0167-4412.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

A full-length cDNA clone encoding microbody **NAD** (+)-dependent ***malate*** **dehydrogenase** (MDH) of cucumber has been isolated. The deduced amino acid sequence is 97% identical to glyoxysomal MDH (gMDH) of watermelon, including the amino terminal putative transit peptide. The cucumber genome contains only a single copy of this gene. Expression of this mdh **gene** increases dramatically in cotyledons during the few days immediately following seed imbibition, in parallel with **genes** ***encoding*** isocitrate lyase (ICL) and malate synthase (MS), two glyoxylate cycle enzymes. The level of MDH, ICL and MS mRNAs then declines, but then MDH mRNA increases again together with that of peroxisomal NAD(+)-dependent hydroxypyruvate reductase (HPR). The mdh gene is also expressed during cotyledon senescence, together with hpr, icl and ms genes. These results indicate that a single **gene encodes** MDH which functions in both glyoxysomes and peroxisomes. In contrast to icl and ms genes, expression of the mdh gene is not activated by incubating detached green cotyledons in the dark, nor is it affected by exogenous sucrose in the incubation medium. The function of this microbody MDH and the regulation of its synthesis are discussed.

☐ L18 ANSWER 1 OF 16 MEDLINE on STN

97392567 MEDLINE Full Text

Extracellular release by Trichomonas vaginalis of a NADP+ dependent malic enzyme involved in pathogenicity.

Author

Addis M F; Rappelli P; Cappuccinelli P; Fiori P L

Corporate Source

Institute of Microbiology and Virology, University of Sassari, Italy.

Source

MICROBIAL PATHOGENESIS, (1997 Jul) 23 (1) 55-61. Journal code: 8606191. ISSN: 0882-4010.

[redacted]

Document Type
Journal; Article; (JOURNAL ARTICLE)
Language
English
Abstract
This report presents evidence showing that Trichomonas vaginalis releases in the extracellular environment a functional form of NADP(+)-dependent malic enzyme. The protein which is likely responsible for the oxidative decarboxilase activity had already been identified in previous studies as P65, one of the five adhesive proteins of the protozoan. The same protein had also been described as AP65 by other authors, which identified it as one of the four surface proteins specifically responsible for binding of the parasite to the target cell in a ligand-receptor fashion. **Gene** characterization studies performed on P65 by different authors revealed that the nucleotide sequences of the **genes coding** for P65 display a striking homology with the ones **coding** for the trichomonad malic enzyme. The experiments performed in this work demonstrate that P65 is secreted and retains its adhesive properties in the extracellular environment, being able to bind both erythrocytes and HeLa cells. Therefore, an oxidative decarboxylase activity assay was performed on T. vaginalis cell-free filtrates, in order to assess if the released P65 displays cathalitic properties. The assay revealed that parasite-free supernatants exhibit an oxidative decarboxylase activity which is NADP(+)-dependent. On the basis of the most recent findings on T. vaginalis pathogenetic mechanism, which involves pH-dependent perforins, a role for the secreted enzyme as part of the system is proposed.

Glycerol 3 phosphate dehydrogenase (NADP-linked not yet found!!)

☐ L20 ANSWER 1 OF 12 MEDLINE on STN

97315159 MEDLINE Full Text

The two isoenzymes for yeast NAD +-dependent glycerol 3 -phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation.

Author
Ansell R; Granath K; Hohmann S; Thevelein J M; Adler L
Corporate Source
Department of General and Marine Microbiology, Gothenburg University, Sweden.
Source
EMBO JOURNAL, (1997 May 1) 16 (9) 2179-87. Journal code: 8208664. ISSN: 0261-4189.
Document Type
Journal; Article; (JOURNAL ARTICLE)
Language
English
Abstract
The two homologous **genes** GPD1 and GPD2 **encode** the isoenzymes of **NAD** -dependent **glycerol 3** - ***phosphate*** **dehydrogenase** in the yeast Saccharomyces cerevisiae. Previous studies showed that GPD1 plays a role in osmoadaptation since its expression is induced by osmotic stress and gpd1 delta mutants are osmosensitive. Here we report that GPD2 has an entirely different physiological role. Expression of GPD2 is not affected by changes in external osmolarity, but is stimulated by anoxic conditions. Mutants lacking GPD2 show poor growth under anaerobic conditions. Mutants deleted for both GPD1 and

GPD2 do not produce detectable glycerol, are highly osmosensitive and fail to grow under anoxic conditions. This growth inhibition, which is accompanied by a strong intracellular accumulation of NADH, is relieved by external addition of acetaldehyde, an effective oxidizer of NADH. Thus, glycerol formation is strictly required as a redox sink for excess cytosolic NADH during anaerobic metabolism. The anaerobic induction of GPD2 is independent of the HOG pathway which controls the osmotic induction of GPD1. Expression of GPD2 is also unaffected by ROX1 and ROX3, **encoding** putative regulators of hypoxic and stress-controlled **gene** expression. In addition, GPD2 is induced under aerobic conditions by the addition of bisulfite which causes NADH accumulation by inhibiting the final, reductive step in ethanol fermentation and this induction is reversed by addition of acetaldehyde. We conclude that expression of GPD2 is controlled by a novel, oxygen-independent, signalling pathway which is required to regulate metabolism under anoxic conditions.

NADP-linked: STILL LOOKING

Glyceraldehyde-3-phosphate dehydrogenase

☐ L28 ANSWER 1 OF 6 MEDLINE on STN

97369819 MEDLINE Full Text

Functional complementation of an Escherichia coli gap mutant supports an amphibolic role for NAD (P)-dependent glyceraldehyde -3 - phosphate dehydrogenase of Synechocystis sp. strain PCC 6803.

Author

Valverde F; Losada M; Serrano A

Corporate Source

Instituto de Bioquimica Vegetal y Fotosintesis, Centro de Investigacion Isla de la Cartuja, Universidad de Sevilla-CSIC, Seville, Spain.

Source

JOURNAL OF BACTERIOLOGY, (1997 Jul) 179 (14) 4513-22. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

The gap-2 **gene** , **encoding** the **NAD** (P)-dependent D- ***glyceraldehyde*** **-3 - phosphate dehydrogenase** (GAPDH2) of the cyanobacterium Synechocystis sp. strain PCC 6803, was cloned by functional complementation of an Escherichia coli gap mutant with a genomic DNA library; this is the first time that this cloning strategy has been used for a GAPDH involved in photosynthetic carbon assimilation. The Synechocystis DNA region able to complement the E. coli gap mutant was narrowed down to 3 kb and fully sequenced. A single complete open reading frame of 1,011 bp ***encoding*** a protein of 337 amino acids was found and identified as the putative gap-2 **gene** identified in the complete genome

[redacted]

sequence of this organism. Determination of the transcriptional start point, identification of putative promoter and terminator sites, and orientation of the truncated flanking genes suggested the gap-2 transcript should be monocystronic, a possibility further confirmed by Northern blot studies. Both natural and recombinant homotetrameric GAPDH2s were purified and found to exhibit virtually identical physicochemical and kinetic properties. The recombinant GAPDH2 showed the dual pyridine nucleotide specificity characteristic of the native cyanobacterial enzyme, and similar ratios of NAD- to NADP-dependent activities were found in cell extracts from Synechocystis as well as in those from the complemented E. coli clones. The deduced amino acid sequence of Synechocystis GAPDH2 presented a high degree of identity with sequences of the chloroplastic NADP-dependent enzymes. In agreement with this result, immunoblot analysis using monospecific antibodies raised against GAPDH2 showed the presence of the 38-kDa GAPDH subunit not only in crude extracts from the gap-2-expressing E. coli clones and all cyanobacteria that were tested but also in those from eukaryotic microalgae and plants. Western and Northern blot experiments showed that gap-2 is conspicuously expressed, although at different levels, in Synechocystis cells grown in different metabolic regimens, even under chemoheterotrophic conditions. A possible amphibolic role of the cyanobacterial GAPDH2, namely, anabolic for photosynthetic carbon assimilation and catabolic for carbohydrate degradative pathways, is discussed.

☐ L30 ANSWER 2 OF 4 MEDLINE on STN

96257768 MEDLINE Full Text

Enzymic and molecular characterization of NADP -dependent glyceraldehyde -3 -phosphate dehydrogenase from Synechococcus PCC 7942: resistance of the enzyme to hydrogen peroxide.

Author

Tamoi M; Ishikawa T; Takeda T; Shigeoka S

Corporate Source

Department of Food and Nutrition, Kinki University, Nara, Japan.

Source

BIOCHEMICAL JOURNAL, (1996 Jun 1) 316 (Pt 2) 685-90. Journal code: 2984726R. ISSN: 0264-6021.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

NADP -dependent **glyceraldehyde -3 -phosphate** ***dehydrogenase*** (GAPDH) has been purified to electrophoretic homogeneity from Synechococcus PCC 7942 cells. The native enzyme had a molecular mass of 160 kDa and consisted of four subunits with a molecular mass of 41 kDa. The activity was 6-fold higher with NADPH than with NADH; the apparent Km values for NADPH and NADH were 62 +/- 4.5 and 420 +/- 10.5 microM respectively. The ***gene*** **encoding** NADP-dependent GAPDH was cloned from the chromosomal DNA of Synechococcus 7942. A 1140 bp open reading frame, encoding an enzyme of 380 amino acid residues (approx.molecular mass of 41.3 kDa) was observed. The deduced amino acid sequence of the gene had a greater sequence similarity to the NADP-dependent and chloroplastic form than to the NAD-dependent and cytosolic form. The Synechococcus 7942 enzyme lacked one of the cysteines involved in the light-

[redacted]

dependent regulation of the chloroplast enzymes of higher plants. The recombinant enzyme expressed in Escherichia coli as well as the native enzyme purified from Synechococcus 7942 cells were resistant to 1 mM H2O2.

Xylose-1 dehydrogenase

```
LOCUS       JC4251                    329 aa            linear   PLN 03-JUN-2002
DEFINITION  D-xylose 1-dehydrogenase (NADP) (EC 1.1.1.179) - yeast
            (Kluyveromyces marxianus var. lactis).
ACCESSION   JC4251
VERSION     JC4251  GI:1364169
DBSOURCE    pir: locus JC4251;

            summary: #length 329 #molecular-weight 37516 #checksum 5185
            ;
            genetic: #gene xyl1 #map_position V
            ;
            superfamily: aldehyde reductase
            ;
            PIR dates: 10-Sep-1999 #sequence_revision 10-Sep-1999 #text_change
            03-Jun-2002
            .
KEYWORDS    NADP; oxidoreductase.
SOURCE      Kluyveromyces lactis
  ORGANISM  Kluyveromyces lactis
            Eukaryota; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes;
            Saccharomycetales; Saccharomycetaceae; Kluyveromyces.
REFERENCE   1  (residues 1 to 329)
  AUTHORS   Billard,P., Menart,S., Fleer,R. and Bolotin-Fukuhara,M.
  TITLE     Isolation and characterization of the gene encoding xylose
            reductase from Kluyveromyces lactis
  JOURNAL   Gene 162 (1), 93-97 (1995)
  MEDLINE   96009884
   PUBMED   7557424
COMMENT     This enzyme is NADPH-dependent and essential for growth on xylose.
FEATURES             Location/Qualifiers
     source          1..329
                     /organism="Kluyveromyces lactis"
                     /db_xref="taxon:28985"
     Protein         1..329
                     /product="D-xylose 1-dehydrogenase (NADP)"
                     /EC_number="1.1.1.179"
ORIGIN
        1 mtylaetvtl nngekmplvg lgcwkmpndv cadqiyeaik igyrlfdgaq dyanekevgq
       61 gvnvaikegl vkredlvvvs klwnsfhhpd nvpralertl sdlqldyvdi fyihfplafk
      121 pvpfdekypp gfytgkedea kghieeeqvp lldtwralek lvdqgkiksl gisnfsgali
      181 qdllrgarik pvalqiehhp yltqerliky vknagiqvva yssfgpvsfl elenkkalnt
      241 ptlfehdtik siaskhkvtp qqvllrwatq ngiaiipkss kkerlldnlr indaltltdd
      301 elkqisglnq nirfndpwew ldnefptfi
```

NOT FOUND THE NAD-LINKED CXyloseYET

Orotate reductase

[redacted]

1: S72324. orotate reductase...[gi:7493932] BLink, Domains, Links

```
LOCUS       S72324                  520 aa            linear   PLN 03-JUN-2002
DEFINITION  orotate reductase (NADH2) (EC 1.3.1.14) - Emericella nidulans.
ACCESSION   S72324
VERSION     S72324  GI:7493932
DBSOURCE    pir: locus S72324;

            summary: #length 520 #molecular-weight 54816 #checksum 1821
            ;
            genetic: #gene PyrE #introns 148/3
            ;
            PIR dates: 04-May-1998 #sequence_revision 15-May-1998 #text_change
            03-Jun-2002
            .
KEYWORDS    oxidoreductase.
SOURCE      Emericella nidulans
  ORGANISM  Emericella nidulans
            Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
            Eurotiales; Trichocomaceae; Emericella.
REFERENCE   1  (residues 1 to 520)
  AUTHORS   Gustafson,G., Davis,G., Waldron,C., Smith,A. and Henry,M.
  TITLE     Identification of a new antifungal target site through a dual
            biochemical and molecular-genetics approach
  JOURNAL   Curr. Genet. 30 (2), 159-165 (1996)
  MEDLINE   96304294
   PUBMED   8660469
FEATURES             Location/Qualifiers
     source          1..520
                     /organism="Emericella nidulans"
                     /db_xref="taxon:162425"
     Protein         1..520
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                     /EC_number="1.3.1.14"
                     /note="dihydroorotate dehydrogenase"
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      121 dtlkmlykyg lhprergdpd gdgalatevf gytlsnpigi sggldkhaei pdplfaigpa
      181 ivevggttp1 pqdgnprprv frlpsqrami nryglnskga dhmaaileqr vrdfayangf
      241 gaydaakqrv ldgeagvppg slqpgkllav qvaknkatpd gdieaikrdy vycvdrvaky
      301 adilvvnvss pntpglrdlq atapltails avvgaaksvn rktkpyvmvk vspdedsdeq
      361 vsgicdavra sgvdgvivgn ttnrrpdpip qgytlpakeq atlketggys gpqlfdrtva
      421 lvaryrsmld aesetagsak dsaatiaqte pgsenvppve apsglprkvi fasggitngk
      481 qahavldtga svammytgvv yggvgtvtrv kqelrtakke
```

Ferredoxin reductase

☐ L102 ANSWER 2 OF 3 MEDLINE on STN

95403362 MEDLINE Full Text

Azotobacter vinelandii NADPH:ferredoxin reductase cloning, sequencing, and overexpression.

Author

Isas J M; Yannone S M; Burgess B K

Corporate Source

Department of Molecular Biology and Biochemistry, University of California, Irvine 92717, USA. CONTRACT NUMBER: RO1-GM45209 (NIGMS)

Source

JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Sep 8) 270 (36) 21258-63. Journal code: 2985121R. ISSN: 0021-9258.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

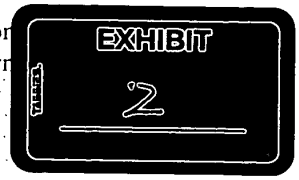
English

Abstract

Azotobacter vinelandii ferredoxin I (AvFdI) controls the expression of another protein that was originally designated Protein X. Recently we reported that Protein X is a NADPH-specific flavoprotein that binds specifically to FdI (Isas, J.M., and Burgess, B.K. (1994) J. Biol. Chemical 269, 19404-19409). The ***gene*** **encoding** this protein has now been cloned and sequenced. Protein X is 33% identical and has an overall 53% similarity with the fpr ***gene*** product from Escherichia coli that **encodes** NADPH: ***ferredoxin*** **reductase.** On the basis of this similarity and the similarity of the physical properties of the two proteins, we now designate Protein X as A. vinelandii NADPH:**ferredoxin reductase** and its gene as the fpr gene. The protein has been overexpressed in its native background in A. vinelandii by using the broad host range multicopy plasmid, pKT230. In addition to being regulated by FdI, the fpr gene product is overexpressed when A. vinelandii is grown under N2-fixing conditions even though the fpr gene is not preceded by a nif specific promoter. By analogy to what is known about fpr expression in E. coli, we propose that FdI may exert its regulatory effect on fpr by interacting with the SoxRS regulon.

NO NAD-LINKED FERREDOXIN REDUCTASE FOUND YET

dependent regulation of the chloroplast enzymes of higher plants. The recor
expressed in Escherichia coli as well as the native enzyme purified from Syn
7942 cells were resistant to 1 mM H2O2.



Xylose-1 dehydrogenase

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LOCUS       JC4251                    329 aa            linear   PLN 03-JUN-2002
DEFINITION  D-xylose 1-dehydrogenase (NADP) (EC 1.1.1.179) - yeast
            (Kluyveromyces marxianus var. lactis).
ACCESSION   JC4251
VERSION     JC4251  GI:1364169
DBSOURCE    pir: locus JC4251;

            summary: #length 329 #molecular-weight 37516 #checksum 5185
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            genetic: #gene xyl1 #map_position V
            ;
            superfamily: aldehyde reductase
            ;
            PIR dates: 10-Sep-1999 #sequence_revision 10-Sep-1999 #text_change
            03-Jun-2002
            .
KEYWORDS    NADP; oxidoreductase.
SOURCE      Kluyveromyces lactis
  ORGANISM  Kluyveromyces lactis
            Eukaryota; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes;
            Saccharomycetales; Saccharomycetaceae; Kluyveromyces.
REFERENCE   1  (residues 1 to 329)
  AUTHORS   Billard,P., Menart,S., Fleer,R. and Bolotin-Fukuhara,M.
  TITLE     Isolation and characterization of the gene encoding xylose
            reductase from Kluyveromyces lactis
  JOURNAL   Gene 162 (1), 93-97 (1995)
  MEDLINE   96009884
   PUBMED   7557424
COMMENT     This enzyme is NADPH-dependent and essential for growth on xylose.
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      181 qdllrgarik pvalqiehhp yltqerliky vknagiqvva yssfgpvsfl elenkkalnt
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```

NOT FOUND THE NAD-LINKED CXyloseYET

Orotate reductase

[redacted]

1: S72324. orotate reductase...[gi:7493932] BLink, Domains, Links

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LOCUS       S72324                  520 aa            linear   PLN 03-JUN-2002
DEFINITION  orotate reductase (NADH2) (EC 1.3.1.14) - Emericella nidulans.
ACCESSION   S72324
VERSION     S72324  GI:7493932
DBSOURCE    pir: locus S72324;

            summary: #length 520 #molecular-weight 54816 #checksum 1821
            ;
            genetic: #gene PyrE #introns 148/3
            ;
            PIR dates: 04-May-1998 #sequence_revision 15-May-1998 #text_change
            03-Jun-2002
            .
KEYWORDS    oxidoreductase.
SOURCE      Emericella nidulans
  ORGANISM  Emericella nidulans
            Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
            Eurotiales; Trichocomaceae; Emericella.
REFERENCE   1  (residues 1 to 520)
  AUTHORS   Gustafson,G., Davis,G., Waldron,C., Smith,A. and Henry,M.
  TITLE     Identification of a new antifungal target site through a dual
            biochemical and molecular-genetics approach
  JOURNAL   Curr. Genet. 30 (2), 159-165 (1996)
  MEDLINE   96304294
   PUBMED   8660469
FEATURES             Location/Qualifiers
     source          1..520
                     /organism="Emericella nidulans"
                     /db_xref="taxon:162425"
     Protein         1..520
                     /product="orotate reductase (NADH2)"
                     /EC_number="1.3.1.14"
                     /note="dihydroorotate dehydrogenase"
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      121 dtlkmlykyg lhprergdpd gdgalatevf gytlsnpigi sggldkhaei pdplfaigpa
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      241 gaydaakqrv ldgeagvppg slqpgkllav qvaknkatpd gdieaikrdy vycvdrvaky
      301 adilvvnvss pntpglrdlq atapltails avvgaaksvn rktkpyvmvk vspdedsdeq
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      481 qahavldtga svammytgvv yggvgtvtrv kqelrtakke
```

Ferredoxin reductase

☐ L102 ANSWER 2 OF 3 MEDLINE on STN

95403362 MEDLINE Full Text

Azotobacter vinelandii NADPH:ferredoxin reductase cloning, sequencing, and overexpression.

Author

Isas J M; Yannone S M; Burgess B K

Corporate Source

[redacted]

Examples of pairs of genes encoding pairs of dehydrogenases with different specificities for NAD and NADP

1. Glutamate dehydrogenase.

☐ L4 ANSWER 1 OF 5 MEDLINE on STN

97113544 MEDLINE Full Text

The NAD(P)H-utilizing glutamate dehydrogenase of Bacteroides thetaiotaomicron belongs to enzyme family I, and its activity is affected by trans-acting gene(s) positioned downstream of gdhA.

Author

Baggio L; Morrison M

Corporate Source

Department of Animal Sciences, University of Nebraska, Lincoln 68583, USA.

Source

JOURNAL OF BACTERIOLOGY, (1996 Dec) 178 (24) 7212-20. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

Previous studies have suggested that regulation of the enzymes of ammonia assimilation in human colonic Bacteroides species is coordinated differently than in other eubacteria. The **gene encoding** an **NAD** (P)H-dependent **glutamate dehydrogenase** (gdhA) in Bacteroides thetaiotaomicron was cloned and expressed in Escherichia coli by mutant complementation from the recombinant plasmid pANS100. Examination of the predicted GdhA amino acid sequence revealed that this enzyme possesses motifs typical of the family I-type hexameric GDH proteins. Northern blot analysis with a gdhA-specific probe indicated that a single transcript with an electrophoretic mobility of approximately 1.6 kb was produced in both B. thetaiotaomicron and E. coli gdhA+ transformants. Although gdhA transcription was unaffected, no GdhA enzyme activity could be detected in E. coli transformants when smaller DNA fragments from pANS100, which contained the entire gdhA gene, were analyzed. Enzyme activity was restored if these E. coli strains were cotransformed with a second plasmid, which contained a 3-kb segment of DNA located downstream of the gdhA coding region. Frameshift mutagenesis within the DNA downstream of gdhA in pANS100 also resulted in the loss of GdhA enzyme activity. Collectively, these results are interpreted as evidence for the role of an additional gene product(s) in modulating the activity of GDH enzyme activity. Insertional mutagenesis experiments which led to disruption of the gdhA gene on the B. thetaiotaomicron chromosome indicated that gdhA mutants were not glutamate auxotrophs, but attempts to isolate similar mutants with insertion mutations in the region downstream of the gdhA gene were unsuccessful.

☐ L6 ANSWER 1 OF 7 MEDLINE on STN

96180651 MEDLINE Full Text

Nucleotide sequence and expression of the gene encoding NADP +- dependent

[redacted]

glutamate dehydrogenase (gdhA) from Agaricus bisporus.

Author

Schaap P J; Muller Y; Baars J J; Op den Camp H J; Sonnenberg A S; van Griensven L J; Visser J

Corporate Source

Section Molecular Genetics of Industrial Microorganisms, Wageningen Agricultural University, The Netherlands.

Source

MOLECULAR AND GENERAL GENETICS, (1996 Feb 25) 250 (3) 339-47. Journal code: 0125036. ISSN: 0026-8925.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

The **gene encoding NADP** +-dependent ***glutamate*** **dehydrogenase** (gdhA) was isolated from an Agaricus bisporus recombinant phage lambda library. The deduced amino acid sequence would specify a 457-amino acid protein that is highly homologous in sequence to those derived from previously isolated and characterized genes coding for microbial NADP+-GDH. The open reading frame is interrupted by six introns. None of the introns is located at either one of the positions of the two introns conserved in the corresponding open reading frames of the ascomycete fungi Aspergillus nidulans and Neurospora crassa. Northern analysis suggests that the A. bisporus gdhA gene is transcriptionally regulated and that, unlike the case in ascomycetes, transcription of this gene is repressed upon the addition of ammonium to the cu

Aldehyde dehdrogenase

☐ L12 ANSWER 1 OF 6 MEDLINE on STN

96134984 MEDLINE Full Text

Cloning and characterization of a gene (msdA) encoding methylmalonic acid semialdehyde dehydrogenase from Streptomyces coelicolor.

Author

Zhang Y X; Tang L; Hutchinson C R

Corporate Source

School of Pharmacy, University of Wisconsin, Madison 53706, USA. CONTRACT NUMBER: GM31925 (NIGMS)

Source

JOURNAL OF BACTERIOLOGY, (1996 Jan) 178 (2) 490-5. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

A homolog of the mmsA **gene** of Pseudomonas aeruginosa, which ***encodes*** methylmalonic acid semialdehyde dehydrogenase (MSDH) and is involved in valine catabolism in pseudomonads and mammals, was cloned and sequenced from Streptomyces coelicolor. Of the two open reading frames (ORFs) found, which are convergently transcribed and separated by a 62-nucleotide noncoding region, the deduced amino acid

sequence of the msdA ORF (homologous to mmsA) is similar to a variety of prokaryotic and eukaryotic **aldehyde** ***dehydrogenases*** that utilize **NAD** +, particularly to the MmsA protein from P. aeruginosa. No significant similarity was found between the deduced product of ORF1 and known proteins in the databases. An S. coelicolor msdA mutant, constructed by insertion of a hygromycin resistance **gene** (hyg) into the msdA **coding** region, lost the MSDH activity and the ability to grow in a minimal medium with valine or isobutyrate as the sole carbon source but grew on propionate. The msdA::hyg mutation was complemented by introduction of the msdA gene on a plasmid. When the S. coelicolor msdA gene was overexpressed in Escherichia coli under the control of the T7 promoter, a protein of 51-kDa, corresponding to the approximate mass of the predicted S. coelicolor msdA product (52.6 kDa), and specific MSDH activity were detected. These results strongly suggest that msdA indeed encodes the MSDH that is involved in valine catabolism in S. coelicolor.

Primary structures of alcohol and aldehyde dehydrogenase genes of Entamoeba histolytica.

Author

Samuelson J; Zhang W W; Kumar A; Descoteaux S; Shen P S; Bailey G

Corporate Source

Department of Tropical Public Health, Harvard School of Public Health, Boston, MA 02115.

Source

ARCHIVES OF MEDICAL RESEARCH, (1992) 23 (2) 31-3. Journal code: 9312706. ISSN: 0188-4409.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

Ethanol is the major metabolic product of glucose fermentation by the protozoan parasite E. histolytica under the anaerobic conditions found in the lumen of the colon. With the goal of finding new targets for anti-amebic drugs, the E. histolytica **NADP** (+)-dependent alcohol dehydrogenase gene (EhADH1; EC 1.1.1.2) and an **aldehyde dehydrogenase** gene (EhALDH1; EC 1.3.2) were cloned. The EhADH1 alcohol dehydrogenase **gene** ***encoded*** -39 kDa protein with 62 and 60% amino acid identities, respectively, with NADP(+)-dependent alcohol dehydrogenases of anaerobic bacteria Thermoanaerobium brockii and Clostridia beijerinckii. In contrast, EhADH1 showed a 15% amino acid identity with the closest human alcohol dehydrogenase. An EhADH1-glutathione-S-transferase fusion protein showed the expected NADP(+)-dependent alcohol dehydrogenase and NADPH-dependent acetaldehyde reductase activities. The enzymatic activities of the EhADH1 fusion protein were inhibited by pyrazole and 4-methyl pyrazole. The E. histolytica aldehyde dehydrogenase EhALDH1 **gene encoded** a 60 kDa protein, which showed a 36% amino acid identity over a 451 amino acid overlap with the human stomach aldehyde dehydrogenase (ALDH3).

Alcohol dehydrogenase

[redacted]

☐ L55 ANSWER 2 OF 17 MEDLINE on STN

96125263 MEDLINE Full Text

Cloning and overexpression in Escherichia coli of the genes encoding NAD - dependent alcohol dehydrogenase from two Sulfolobus species.

Author

Cannio R; Fiorentino G; Carpinelli P; Rossi M; Bartolucci S

Corporate Source

Dipartimento di Chimica Organica e Biologica, Universita degli Studi di Napoli Federico II, Italy.

Source

JOURNAL OF BACTERIOLOGY, (1996 Jan) 178 (1) 301-5. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

The **gene** adh **encoding** a **NAD** -dependent ***alcohol*** **dehydrogenase** from the novel strain RC3 of Sulfolobus sp. was cloned and sequenced. Both the adh gene from Sulfolobus sp. strain RC3 and the alcohol dehydrogenase gene from Sulfolobus solfataricus (DSM 1617) were expressed at a high level in Escherichia coli, and the recombinant enzymes were purified, characterized, and compared. Only a few amino acid replacements were responsible for the different kinetic and physicochemical features investigated.

☐ L14 ANSWER 1 OF 5 MEDLINE on STN

97352709 MEDLINE Full Text

Purification and sequence analysis of a novel NADP (H)-dependent type III alcohol dehydrogenase from Thermococcus strain AN1.

Author

Li D; Stevenson K J

Corporate Source

Department of Biological Sciences, The University of Calgary, Alberta, Canada.

Source

JOURNAL OF BACTERIOLOGY, (1997 Jul) 179 (13) 4433-7. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

An **NADP** (H)-dependent **alcohol dehydrogenase** was isolated from the hyperthermophilic archaeon Thermococcus strain AN1. This enzyme is a homotetramer with a subunit molecular weight of 46,700. The enzyme oxidizes a series of primary linear alcohols but not methanol. The pH and temperature optima with ethanol as the substrate are 6.8 to 7.0 and 85 degrees C, respectively. The enzyme readily reduced acetaldehyde with NADPH as the cofactor. The **gene encoding** this enzyme has been cloned and sequenced. An open reading frame of 1,218 bp, starting with ATG and ending with TGA, was identified and corresponded to 406 amino acids. Sequence comparisons show that this Thermococcus strain AN1 enzyme has significant homologies with enzymes from the newly defined type

III alcohol dehydrogenase family. Thermococcus strain AN1 alcohol dehydrogenase is the first archaeal enzyme belonging to this family.

Malate dehydrogenase

☐ L16 ANSWER 4 OF 7 MEDLINE on STN

95161707 MEDLINE Full Text

Expression of a single gene encoding microbody NAD -malate dehydrogenase during glyoxysome and peroxisome development in cucumber.

Author

Kim D J; Smith S M

Corporate Source

Institute of Cell and Molecular Biology, University of Edinburgh, UK.

Source

PLANT MOLECULAR BIOLOGY, (1994 Dec) 26 (6) 1833-41. Journal code: 9106343. ISSN: 0167-4412.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

A full-length cDNA clone encoding microbody **NAD** (+)-dependent ***malate*** **dehydrogenase** (MDH) of cucumber has been isolated. The deduced amino acid sequence is 97% identical to glyoxysomal MDH (gMDH) of watermelon, including the amino terminal putative transit peptide. The cucumber genome contains only a single copy of this gene. Expression of this mdh **gene** increases dramatically in cotyledons during the few days immediately following seed imbibition, in parallel with **genes** ***encoding*** isocitrate lyase (ICL) and malate synthase (MS), two glyoxylate cycle enzymes. The level of MDH, ICL and MS mRNAs then declines, but then MDH mRNA increases again together with that of peroxisomal NAD(+)-dependent hydroxypyruvate reductase (HPR). The mdh gene is also expressed during cotyledon senescence, together with hpr, icl and ms genes. These results indicate that a single **gene encodes** MDH which functions in both glyoxysomes and peroxisomes. In contrast to icl and ms genes, expression of the mdh gene is not activated by incubating detached green cotyledons in the dark, nor is it affected by exogenous sucrose in the incubation medium. The function of this microbody MDH and the regulation of its synthesis are discussed.

☐ L18 ANSWER 1 OF 16 MEDLINE on STN

97392567 MEDLINE Full Text

Extracellular release by Trichomonas vaginalis of a NADP+ dependent malic enzyme involved in pathogenicity.

Author

Addis M F; Rappelli P; Cappuccinelli P; Fiori P L

Corporate Source

Institute of Microbiology and Virology, University of Sassari, Italy.

Source

MICROBIAL PATHOGENESIS, (1997 Jul) 23 (1) 55-61. Journal code: 8606191. ISSN: 0882-4010.

Document Type
Journal; Article; (JOURNAL ARTICLE)
Language
English
Abstract
This report presents evidence showing that Trichomonas vaginalis releases in the extracellular environment a functional form of NADP(+)-dependent malic enzyme. The protein which is likely responsible for the oxidative decarboxilase activity had already been identified in previous studies as P65, one of the five adhesive proteins of the protozoan. The same protein had also been described as AP65 by other authors, which identified it as one of the four surface proteins specifically responsible for binding of the parasite to the target cell in a ligand-receptor fashion. **Gene** characterization studies performed on P65 by different authors revealed that the nucleotide sequences of the **genes coding** for P65 display a striking homology with the ones **coding** for the trichomonad malic enzyme. The experiments performed in this work demonstrate that P65 is secreted and retains its adhesive properties in the extracellular environment, being able to bind both erythrocytes and HeLa cells. Therefore, an oxidative decarboxylase activity assay was performed on T. vaginalis cell-free filtrates, in order to assess if the released P65 displays cathalitic properties. The assay revealed that parasite-free supernatants exhibit an oxidative decarboxylase activity which is NADP(+)-dependent. On the basis of the most recent findings on T. vaginalis pathogenetic mechanism, which involves pH-dependent perforins, a role for the secreted enzyme as part of the system is proposed.

Glycerol 3 phosphate dehydrogenase (NADP-linked not yet found!!)

☐ L20 ANSWER 1 OF 12 MEDLINE on STN

97315159 MEDLINE Full Text

The two isoenzymes for yeast NAD +-dependent glycerol 3 -phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation.

Author
Ansell R; Granath K; Hohmann S; Thevelein J M; Adler L
Corporate Source
Department of General and Marine Microbiology, Gothenburg University, Sweden.
Source
EMBO JOURNAL, (1997 May 1) 16 (9) 2179-87. Journal code: 8208664. ISSN: 0261-4189.
Document Type
Journal; Article; (JOURNAL ARTICLE)
Language
English
Abstract
The two homologous **genes** GPD1 and GPD2 **encode** the isoenzymes of **NAD** -dependent **glycerol 3** - ***phosphate*** **dehydrogenase** in the yeast Saccharomyces cerevisiae. Previous studies showed that GPD1 plays a role in osmoadaptation since its expression is induced by osmotic stress and gpd1 delta mutants are osmosensitive. Here we report that GPD2 has an entirely different physiological role. Expression of GPD2 is not affected by changes in external osmolarity, but is stimulated by anoxic conditions. Mutants lacking GPD2 show poor growth under anaerobic conditions. Mutants deleted for both GPD1 and

[redacted]

GPD2 do not produce detectable glycerol, are highly osmosensitive and fail to grow under anoxic conditions. This growth inhibition, which is accompanied by a strong intracellular accumulation of NADH, is relieved by external addition of acetaldehyde, an effective oxidizer of NADH. Thus, glycerol formation is strictly required as a redox sink for excess cytosolic NADH during anaerobic metabolism. The anaerobic induction of GPD2 is independent of the HOG pathway which controls the osmotic induction of GPD1. Expression of GPD2 is also unaffected by ROX1 and ROX3, **encoding** putative regulators of hypoxic and stress-controlled **gene** expression. In addition, GPD2 is induced under aerobic conditions by the addition of bisulfite which causes NADH accumulation by inhibiting the final, reductive step in ethanol fermentation and this induction is reversed by addition of acetaldehyde. We conclude that expression of GPD2 is controlled by a novel, oxygen-independent, signalling pathway which is required to regulate metabolism under anoxic conditions.

NADP-linked: STILL LOOKING

Glyceraldehyde-3-phosphate dehydrogenase

☐ L28 ANSWER 1 OF 6 MEDLINE on STN

97369819 MEDLINE Full Text

Functional complementation of an Escherichia coli gap mutant supports an amphibolic role for NAD (P)-dependent glyceraldehyde -3 - phosphate dehydrogenase of Synechocystis sp. strain PCC 6803.

Author

Valverde F; Losada M; Serrano A

Corporate Source

Instituto de Bioquimica Vegetal y Fotosintesis, Centro de Investigacion Isla de la Cartuja, Universidad de Sevilla-CSIC, Seville, Spain.

Source

JOURNAL OF BACTERIOLOGY, (1997 Jul) 179 (14) 4513-22. Journal code: 2985120R. ISSN: 0021-9193.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

The gap-2 **gene** , **encoding** the **NAD** (P)-dependent D- ***glyceraldehyde*** **-3** - **phosphate dehydrogenase** (GAPDH2) of the cyanobacterium Synechocystis sp. strain PCC 6803, was cloned by functional complementation of an Escherichia coli gap mutant with a genomic DNA library; this is the first time that this cloning strategy has been used for a GAPDH involved in photosynthetic carbon assimilation. The Synechocystis DNA region able to complement the E. coli gap mutant was narrowed down to 3 kb and fully sequenced. A single complete open reading frame of 1,011 bp ***encoding*** a protein of 337 amino acids was found and identified as the putative gap-2 **gene** identified in the complete genome

[redacted]

sequence of this organism. Determination of the transcriptional start point, identification of putative promoter and terminator sites, and orientation of the truncated flanking genes suggested the gap-2 transcript should be monocystronic, a possibility further confirmed by Northern blot studies. Both natural and recombinant homotetrameric GAPDH2s were purified and found to exhibit virtually identical physicochemical and kinetic properties. The recombinant GAPDH2 showed the dual pyridine nucleotide specificity characteristic of the native cyanobacterial enzyme, and similar ratios of NAD- to NADP-dependent activities were found in cell extracts from Synechocystis as well as in those from the complemented E. coli clones. The deduced amino acid sequence of Synechocystis GAPDH2 presented a high degree of identity with sequences of the chloroplastic NADP-dependent enzymes. In agreement with this result, immunoblot analysis using monospecific antibodies raised against GAPDH2 showed the presence of the 38-kDa GAPDH subunit not only in crude extracts from the gap-2-expressing E. coli clones and all cyanobacteria that were tested but also in those from eukaryotic microalgae and plants. Western and Northern blot experiments showed that gap-2 is conspicuously expressed, although at different levels, in Synechocystis cells grown in different metabolic regimens, even under chemoheterotrophic conditions. A possible amphibolic role of the cyanobacterial GAPDH2, namely, anabolic for photosynthetic carbon assimilation and catabolic for carbohydrate degradative pathways, is discussed.

☐ L30 ANSWER 2 OF 4 MEDLINE on STN

96257768 MEDLINE Full Text

Enzymic and molecular characterization of NADP -dependent glyceraldehyde -3 -phosphate dehydrogenase from Synechococcus PCC 7942: resistance of the enzyme to hydrogen peroxide.

Author

Tamoi M; Ishikawa T; Takeda T; Shigeoka S

Corporate Source

Department of Food and Nutrition, Kinki University, Nara, Japan.

Source

BIOCHEMICAL JOURNAL, (1996 Jun 1) 316 (Pt 2) 685-90. Journal code: 2984726R. ISSN: 0264-6021.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

NADP -dependent **glyceraldehyde -3 -phosphate** ***dehydrogenase*** (GAPDH) has been purified to electrophoretic homogeneity from Synechococcus PCC 7942 cells. The native enzyme had a molecular mass of 160 kDa and consisted of four subunits with a molecular mass of 41 kDa. The activity was 6-fold higher with NADPH than with NADH; the apparent Km values for NADPH and NADH were 62 +/- 4.5 and 420 +/- 10.5 microM respectively. The ***gene*** **encoding** NADP-dependent GAPDH was cloned from the chromosomal DNA of Synechococcus 7942. A 1140 bp open reading frame, encoding an enzyme of 380 amino acid residues (approx.molecular mass of 41.3 kDa) was observed. The deduced amino acid sequence of the gene had a greater sequence similarity to the NADP-dependent and chloroplastic form than to the NAD-dependent and cytosolic form. The Synechococcus 7942 enzyme lacked one of the cysteines involved in the light-

[redacted]

dependent regulation of the chloroplast enzymes of higher plants. The recombinant enzyme expressed in Escherichia coli as well as the native enzyme purified from Synechococcus 7942 cells were resistant to 1 mM H2O2.

Xylose-1 dehydrogenase

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LOCUS       JC4251                    329 aa            linear   PLN 03-JUN-2002
DEFINITION  D-xylose 1-dehydrogenase (NADP) (EC 1.1.1.179) - yeast
            (Kluyveromyces marxianus var. lactis).
ACCESSION   JC4251
VERSION     JC4251  GI:1364169
DBSOURCE    pir: locus JC4251;

            summary: #length 329 #molecular-weight 37516 #checksum 5185
            ;
            genetic: #gene xyl1 #map_position V
            ;
            superfamily: aldehyde reductase
            ;
            PIR dates: 10-Sep-1999 #sequence_revision 10-Sep-1999 #text_change
            03-Jun-2002
            .
KEYWORDS    NADP; oxidoreductase.
SOURCE      Kluyveromyces lactis
  ORGANISM  Kluyveromyces lactis
            Eukaryota; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes;
            Saccharomycetales; Saccharomycetaceae; Kluyveromyces.
REFERENCE   1  (residues 1 to 329)
  AUTHORS   Billard,P., Menart,S., Fleer,R. and Bolotin-Fukuhara,M.
  TITLE     Isolation and characterization of the gene encoding xylose
            reductase from Kluyveromyces lactis
  JOURNAL   Gene 162 (1), 93-97 (1995)
  MEDLINE   96009884
   PUBMED   7557424
COMMENT     This enzyme is NADPH-dependent and essential for growth on xylose.
FEATURES             Location/Qualifiers
     source          1..329
                     /organism="Kluyveromyces lactis"
                     /db_xref="taxon:28985"
     Protein         1..329
                     /product="D-xylose 1-dehydrogenase (NADP)"
                     /EC_number="1.1.1.179"
ORIGIN
        1 mtylaetvtl nngekmplvg lgcwkmpndv cadqiyeaik igyrlfdgaq dyanekevgq
       61 gvnraikegl vkredlvvvs klwnsfhhpd nvpralertl sdlqldyvdi fyihfplafk
      121 pvpfdekypp gfytgkedea kghieeeqvp lldtwralek lvdqgkiksl gisnfsgali
      181 qdllrgarik pvalqiehhp yltqerliky vknagiqvva yssfgpvsfl elenkkalnt
      241 ptlfehdtik siaskhkvtp qqvllrwatq ngiaiipkss kkerlldnlr indaltltdd
      301 elkqisglnq nirfndpwew ldnefptfi
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NOT FOUND THE NAD-LINKED CXyloseYET

Orotate reductase

1: S72324. orotate reductase...[gi:7493932] BLink, Domains, Links

```
LOCUS       S72324                    520 aa            linear   PLN 03-JUN-2002
DEFINITION  orotate reductase (NADH2) (EC 1.3.1.14) - Emericella nidulans.
ACCESSION   S72324
VERSION     S72324  GI:7493932
DBSOURCE    pir: locus S72324;

            summary: #length 520 #molecular-weight 54816 #checksum 1821
            ;
            genetic: #gene PyrE #introns 148/3
            ;
            PIR dates: 04-May-1998 #sequence_revision 15-May-1998 #text_change
            03-Jun-2002
            .
KEYWORDS    oxidoreductase.
SOURCE      Emericella nidulans
  ORGANISM  Emericella nidulans
            Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
            Eurotiales; Trichocomaceae; Emericella.
REFERENCE   1  (residues 1 to 520)
  AUTHORS   Gustafson,G., Davis,G., Waldron,C., Smith,A. and Henry,M.
  TITLE     Identification of a new antifungal target site through a dual
            biochemical and molecular-genetics approach
  JOURNAL   Curr. Genet. 30 (2), 159-165 (1996)
  MEDLINE   96304294
   PUBMED   8660469
FEATURES             Location/Qualifiers
     source          1..520
                     /organism="Emericella nidulans"
                     /db_xref="taxon:162425"
     Protein         1..520
                     /product="orotate reductase (NADH2)"
                     /EC_number="1.3.1.14"
                     /note="dihydroorotate dehydrogenase"
ORIGIN
        1 matnsfrklt fsgasrlggc rrlpltcrql rfasdsgaaa attkataesa aesasinvke
       61 apkkagrglr rtvlgtslal tllvgyvygt dtrasvhryg vvpliralyp daedahhigv
      121 dtlkmlykyg lhprergdpd gdgalatevf gytlsnpigi sggldkhaei pdplfaigpa
      181 ivevggttpl pqdgnprprv frlpsqrami nryglnskga dhmaaileqr vrdfayangf
      241 gaydaakqrv ldgeagvppg slqpgkllav qvaknkatpd gdieaikrdy vycvdrvaky
      301 adilvvnvss pntpglrdlq atapltails avvgaaksvn rktkpyvmvk vspdedsdeq
      361 vsgicdavra sgvdgvivgn ttnrrpdpip qgytlpakeq atlketggys gpqlfdrtva
      421 lvaryrsmld aesetagsak dsaatiaqte pgsenvppve apsglprkvi fasggitngk
      481 qahavldtga svammytgvv yggvgtvtrv kqelrtakke
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Ferredoxin reductase

☐ L102 ANSWER 2 OF 3 MEDLINE on STN

95403362 MEDLINE Full Text

Azotobacter vinelandii NADPH:ferredoxin reductase cloning, sequencing, and overexpression.

Author

Isas J M; Yannone S M; Burgess B K

Corporate Source

[redacted]

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Source

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Abstract

Azotobacter vinelandii ferredoxin I (AvFdI) controls the expression of another protein that was originally designated Protein X. Recently we reported that Protein X is a NADPH-specific flavoprotein that binds specifically to FdI (Isas, J.M., and Burgess, B.K. (1994) J. Biol. Chemical 269, 19404-19409). The ***gene*** **encoding** this protein has now been cloned and sequenced. Protein X is 33% identical and has an overall 53% similarity with the fpr ***gene*** product from Escherichia coli that **encodes** NADPH: ***ferredoxin*** **reductase.** On the basis of this similarity and the similarity of the physical properties of the two proteins, we now designate Protein X as A. vinelandii NADPH:**ferredoxin reductase** and its gene as the fpr gene. The protein has been overexpressed in its native background in A. vinelandii by using the broad host range multicopy plasmid, pKT230. In addition to being regulated by FdI, the fpr gene product is overexpressed when A. vinelandii is grown under N2-fixing conditions even though the fpr gene is not preceded by a nif specific promoter. By analogy to what is known about fpr expression in E. coli, we propose that FdI may exert its regulatory effect on fpr by interacting with the SoxRS regulon.

NO NAD-LINKED FERREDOXIN REDUCTASE FOUND YET